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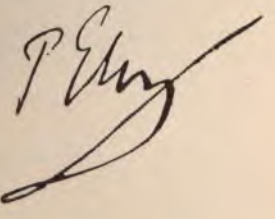

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ON

SPECIFIC THERAPEUTICS

BY

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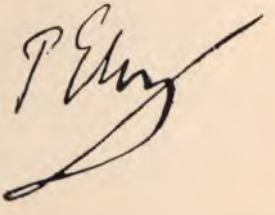

PREFACE.

It was with very sincere pleasure that I accepted the invitation of the Council of the Royal Institute of Public Health with which they honored me, to deliver the Harben Lectures in 1907, for many relationships both of a scientific and social character have always contributed to render a visit to England one of stimulus and recuperation.

The subjects which I chose for my lectures are closely related with those questions, with the study of which I have been connected during the past ten years, viz., the theories of Immunity and Atrepsy, which latter is connected with my researches on Cancer, and the theory of Chemico-therapeutics which I have enunciated in connection with my work on the Trypanosomes.

I am publishing these lectures with a feeling of sincere gratitude for the kind and hospitable reception which I received in London from my friends and colleagues, and especially am I anxious to express my indebtedness to Professor William R. Smith for all his kindness, which rendered my visit so enjoyable. I would also desire to thank Dr. Carl Prausnitz for his very valuable help, and Professor R. Tanner Hewlett for kindly revising the English translation of the Lectures.

P. EHRLICH.

FRANKFORT,
April 22, 1908.

EXPERIMENTAL RESEARCHES ON SPECIFIC THERAPEUTICS.

LECTURE I.

On Immunity with Especial Reference to the Relations Existing between the Distribution and the Action of Antigens.

There can be no doubt that the three great fields of knowledge, Pharmacology, Toxicology and Therapeutics, in their theoretical and practical aspects form the most important branches of medicine. It is matter, therefore, for no surprise that in the study of the various substances with which these sciences are concerned, the mode of action and the reasons for such call for much consideration, and theory and speculation necessarily form a great part of our study.

Besides, pharmacology has but just emerged from the stage of pure observation and description. One was content formerly with describing the physiological effects and the secondary action of substances which act pharmaco-dynamically, as well as the morphological changes which they bring about in the organs and tissues of the body. Observations made on an empirical basis such as this, formed a mass of most needful knowledge, and even to-day we have no hesitation in admitting that the study of the symptomatology of drugs is still a work of absolute necessity and must yield very fruitful results. Indeed, by such means we learn not only how to make use of known drugs in a purposeful manner, but also how to avoid their undesired secondary actions. But merely to increase the contents of our pharmacopœia is not to add to our resources in this desirable direction: for such an increase may depend on accidents, which, in their turn, may be the outcome of empiricism. It is to the great influence which chemistry exerts on medical science that we owe the change in this state of affairs; for it is especially necessary to have clear ideas of the relations between chemical constitution and pharmacological action.

About the middle of the last century the influence of these inquiries made itself especially felt, and this influence is chiefly evident in the mass of drugs with which the united efforts of synthetic chemistry and pharmacology have enriched us. But observers were content with an advance in this direction, based on rational grounds. They recognised a limited number of atom-groupings, which were of importance either for their therapeutic or their toxic action; but the drugs used were directed not against the causes of disease but against the symptoms to which these gave rise; it was not the causes but their effects which were combatted. Therapeutics were chiefly symptomatic, and so it is in many cases to-day. Since the search after the seat and cause of disease has, from the time of Morgagni, and especially under the leadership of Virchow's genius, influenced our entire field of thought, the effect of these considerations has become more evident in our treatment. The features of an ætiological treatment, directed against the causes and the seat of disease, were not satisfactorily brought out by merely insisting on the relationship which existed between the constitution of drugs and their action; the fact was overlooked that between chemical consti-

tution and pharmacological action another and important bond of union exists, which influences the relations between the pharmaco-dynamic agent and the substance on which it is intended to act. This bond of union is the mode of distribution, and represents the sum of the peculiarities of the cells and tissues and of the drug. In this we have to do with a principle so obvious that it should at once be accepted as an axiom, but even when accepted as an axiom it is scarcely ever applied to the study of practical questions. The reason for this, I think, lies partly in a certain disinclination to attempt to master the difficulties of the problem, but mainly in the fact that, in view of the triumphs of synthetic chemistry, *per se*, the biological factor of the pharmaco-dynamic action is somewhat lost sight of. I may mention that from the beginning, as the result of my studies on dyes, I have endeavoured to point out the necessity of the study of localisation; in a word, to give pharmacology an ætiologically therapeutic tendency; regarding the details of this I shall speak further in my second lecture. Excepting the case of dyes, which, by reason of their easily appreciable *properties allow* their distribution in the organism

to be followed, the study of the laws which govern distribution is exceedingly difficult. Besides, owing to the great number of chemicals at our disposal, a large amount of empirical work must be carried out before we are able to find those substances which in any given case will give the desired mode of distribution. All the greater, then, must be our admiration for the powers of nature, in view of the fact that the living organism, when it takes upon itself the production of curative agents, does this in such a manner as to form ideal ætiological remedies. The protective substances of the blood, with which this lecture is concerned, completely fulfil the requirements of the case, and the study of *antigens* and *antibodies* may form the basis of the relationships which must exist between constitution, distribution, and action, in order that our treatment may be successful.

Since the conditions of substances treated of in the study of immunity are specially clear and matters of common knowledge, I shall begin the discussion of my views regarding distribution and localisation with the consideration of this class of phenomena of pharmaco-dynamic and toxicological action.

The discovery of antitoxins by von Behring,

fundamental in itself, has opened to pharmacology and therapeutics this new field in which the principle of distribution is exemplified in an ideal manner: for antitoxins and antibacterial substances are, so to speak, charmed bullets which strike only those objects for whose destruction they have been produced by the organism. I call these substances monotropic; the monotropism of these antibodies is characterised by the fact that they are bacteriotropic or generally speaking ætiotropic, *i.e.*, they are directed against bacteria or against those products of their metabolism (toxins) which cause disease. The definition of monotropism is, therefore, here overlapped by that of specificity, which, in the language of the study of immunity, is the characteristic of monotropic action. As the cause of this specificity we must note, in my opinion, only the effect of chemical relations which exist between the agents of infection, or their products, and the antibodies. From the very beginning my standpoint has been, that all those substances which have the power to bring about the creation of antibodies, I mean the antigens, must be distinguished, as a matter of principle, from the other pharmaco-dynamical and *poisonous substances*. That distinction I con-

sider to be of the greatest importance, and this is further borne out by the fact that in spite of the most strenuous endeavours we have been unable to find any antigen of a known chemical constitution.

I believe that the absorption of antigen-like substances by the body is a phenomenon which bears a close resemblance to the assimilation of nutritive substances. In the case of the other poisonous substances we find more simple phenomena as causes of localisation, but, in my opinion, the powers which influence the distribution of toxins and kindred substances, belong to the domain of chemical synthesis. There has been a tendency of late to bring the colloid nature of immune substances into the foreground, and thus the impression is conveyed that the whole subject of these phenomena might be explained on the ground of the substances being colloid. Against this view it seems to me to be necessary to insist upon the fact that colloid nature and chemical reactive power do not exclude one another; for colloids possess, just as other substances do, certain groupings of atoms which render them capable of reactions of a synthetic nature. Thus, one may introduce into certain aromatic nuclei of

the protein molecule chemical atom-groups, *e.g.*, the nitro-, the amido-, etc., or, on the other hand, the reactive power of any such groups as may be present can be inhibited, as by the removal of the amid. I would here remind you of the researches of Obermayer and Pick, which show that by purely chemical substitution of this kind one may also profoundly alter the antigenic character of albuminous substances, so that in place of specificity for a kind ("Artspecifität") we have a specificity of chemical constitution. I would remind you also of the behaviour of certain colloid dyes—for example, chromazon-red, which possesses all the properties of the azo-dyes, but differs from them in possessing an aldehyde group; it is thus able to react with hydrazin, and by union therewith to be transformed into a hydrazon of blue colour. Besides, I do not believe that for the explanation of such reactions, especially of their specific nature, a few analogies drawn from certain phenomena of the hitherto obscure chemistry of colloids are sufficient; and as a matter of fact it seems to me that the ever-increasing endeavour to build causal relationships on the ground of purely formal analogies, is but little *fitted to advance* our knowledge. The condi-

tion therefore necessary for such action is the presence of two groups, whose chemical relationship is of the closest, and whose interaction is therefore the condition of their union. This axiom as to union is the basis of my side-chain theory.

Let us consider in the first place the action of comparatively simple toxins, which differ in the phenomena of intoxication to which they give rise; the action of the diphtheria-toxin, for example, is absolutely different from that of the tetanus-toxin. In the case of toxins, too, we are justified in assuming a connection between their chemical constitution and action, just as in the case of poisons of known chemical constitution; this connection can in many cases be proved. As an instance of this latter I would note the fact that in the cocaine series it is the residue of benzoyl which causes their anæsthesiophoric character; that the soporific action of certain disulphons is entirely due to the presence in them of ethyl groups; and that the dulcific character of certain sweet substances, *e.g.*, phenetidin-urea, is due to a like group. In the case of the toxins there is a difficulty in our way, due to the fact that, up till now, they have *not lent* themselves to chemical analysis; but

in their case, too, it has been found to be a fruitful heuristic principle to formulate for them similar relations between their constitution and their action. Thus I designate that group of the toxin molecule which is the cause of its peculiar poisonous action as its toxophoric group; but the presence of this toxophoric group is not of itself sufficient to bring about the poisonous action—for whilst the guinea-pig is exceedingly sensitive to the tetanus toxin, the rabbit possesses a relative immunity. The cause of such difference we must therefore attribute to the distribution or localisation of the toxin.

When the poison and the organs sensitive to it do not come in contact, or when sensitiveness of the organs does not exist, the action remains absent.

If we assume those peculiarities of the toxins which cause their distribution to be localised in a special group of the toxin molecule, and the power of the organs and tissues to react with the toxin to be localised in special atom groups of the protoplasm, we arrive at the basis of my side-chain theory. The distributive group of the toxins I call the "haptophoric group," and *the corresponding* chemical organs of the proto-

plasm the "receptors." The relations between the receptors and the haptophoric groups represent the conditions under which distribution takes place. The toxic action can occur only when receptors fitted to anchor the toxins are present.

The existence of receptors can be proved experimentally. For, if to a solution of toxin there be added receptors suitable for anchoring the toxin, the solution becomes non-poisonous; this "binding experiment" plays a great part in the study of immunity, since Wassermann, working on the basis of the conclusions to be drawn from the side-chain theory, first showed that those cells of the central nervous system, which were known to be affected by the action of tetanus-toxin, anchored the toxin.

In order that the poisonous action may take place, we must presume not only the presence of receptors, but that they should be present in positions favourable to the toxin action being brought about. When both the receptors and the organs sensitive to the action of the poison are present, the conditions for infection are naturally most favourable; then distribution takes place at once, the poison circulating until it reaches the sensitive cell. The action of the tetanus-toxin, in the case of the guinea-pig, is

monotropic, for receptors for the tetanus spasmin are present only in the central nervous system; on the other hand, in the rabbit, suitable receptors are present not only in the central nervous system, but also in other organs and in the connective tissue, and the type of distribution in this animal is more complex, and depends upon the point of entrance of the infection or injection. Thus the varying sensitiveness of different species to the same toxin may be explained. The localisation of the receptors is, then, of great moment for the distribution of toxin in the organism, and consequently for its sensitiveness to poisonous action. One may therefore, in general, distinguish four different types of distribution:

(1) In which fitting receptors are not present, the animal possessing natural immunity (the formation of antibodies cannot take place).

(2) In which receptors are present, but only in those organs on which the poison does not act, or in organs of lesser importance; here, again, the animal possesses natural immunity (antibodies may be formed, and immunisation is easily carried out).

(3) In which the receptors are distributed *over various* parts of the organism, and are

present in the organs which are sensitive to the action of the poison. Here a relative immunity exists, and the possibility of infection depends chiefly upon the manner in which the poison is introduced (the conditions for antibody formation are present, and those for immunisation are more or less favourable).

(4) In which the receptors are present only in the organs which are sensitive to the poison; in this case the organism is exceedingly sensitive to the poison (antibody formation is possible; but immunisation is difficult to carry out, and must be begun with small doses, or with weakened poisons).

I think that this systematic division clearly shows the possibilities of distribution and localisation, as well as the conditions under which a poisonous action can take place, and what must be the aim of antitoxic treatment, viz., the alteration of the natural conditions of distribution and the interference with the action of the poison.

In my view, which is based on extensive experience in experimental research, the antitoxins are purely and simply receptors fitted for union with the poisons which have entered the *circulation*. When, for therapeutic or prophy-

lactic purposes we inject an antitoxic serum, the number of receptors in the organism fitted for union with the poison is increased, the quantity of receptors introduced being characterised by the fact that, as antitoxins, they represent dissolved cell contituents, which, by their union with the toxin, can do no harm, but by their presence in considerable quantity bring about a marked change in the conditions of distribution in the organism. The receptors of the organs which are sensitive to the action of the poison are reinforced by a large number of free antitoxin receptors, which, by reason of the factor of distribution, and also because they take up the toxin in the juices of the organism, bring about this condition of affairs. The toxin is thus either kept away from the organs which it threatens to attack, or it comes in contact with them only in an inconsiderable amount, and so the disease runs a favourable course. Of course the toxin, which has already become united to the cell, and has exerted a deleterious influence upon it, cannot be rendered innocuous; for we know that in all reactions of a similar nature observed in the study of immunity, primary union is followed by a stage of secondary consolidation, a stage in which, even

by the addition of a large number of receptors of the needed description, we cannot release the toxin from the union. We see, then, the limitations of antitoxic treatment, which is, in the true sense of the term, a distributive treatment; the antitoxins are really specific drugs by using which we run no risk of harming the component parts of the organism, but which, thanks to their specific monotropism, influence the toxic agent alone. The relations which exist between constitution and action are therefore in no sense influenced by the action of the antitoxins. The toxin molecule, which is anchored by the antitoxin, still possesses a toxophoric group, which, if it could but obtain a suitable localisation, would immediately develop its characteristic action.

I have always held the view that the antitoxins do not in any way destroy the toxin, but that they merely limit its sphere of action by combining with it. If further proof of the correctness of this view be required, we have it in the researches carried out by Morgenroth, who showed that in suitable cases the toxin may be entirely regained from a perfectly neutral union of toxin and antitoxin, just as glucosides may by suitable treatment be resolved

into their two components. The antitoxin, then, exercises its curative influence merely by anchoring the distributive group of the toxins.

When we speak of monotropism or specificity in the case of antigens and antibodies, we of course mean only the chemical relations between haptophoric groups and receptors. For example, toxins may be specific and yet act upon the cells of all species of animals, if the toxin-anchoring receptors be widespread amongst them. The other extreme we find in those cases in which the organism itself reacts to elements introduced into it and forms substances, antibodies, which act on the corresponding antigens; we are here dealing with antibodies which are specifically monotropic, just as the antitoxins are, and which affect only those substances to which they owe their origin. The only difference is that the antitoxins act by localisation alone—when they have anchored the toxins their work is done. The other antibodies, it is true, act at first in a similar way on the substances sensitive to them; but they have a further action on the anchored substances, an action which is either direct, as in those cases (agglutinins and precipitins) in which, like the toxins, they have special ergophoric groups, or indirect, in that

the union is merely a preliminary to their further action on their prey. Thus one class of these antibodies has the power of rendering the cells assimilable by phagocytes (opsonins, bacteriotropic substances); another class (amboceptors) has the power of rendering the cells liable to the action of toxin-like constituents of the blood serum (complements). In the latter case by the simultaneous action of two substances a destructive effect is produced. These substances are also called cytotoxins. For the study of these the way has been prepared by the work of Pfeiffer, Metchnikoff, and Bordet, and by the discovery of Metchnikoff and Bordet that hæmolysins are produced by immunisation against blood corpuscles.

These hæmolysins are of special importance in considering the question of the relations between constitution, distribution, and action, because, in their case, the haptophoric and toxophoric groups are distinct, distribution and toxic effect being dependent upon two different substances, the more stable amboceptor controlling the distribution, and the labile complement the toxic effect. The complement has no direct relations with the cell, on which it acts only through the medium of the amboceptor.

It is a normal constituent of the blood-serum, and its quantity undergoes no change as a result of the process of immunisation. On the other hand the amboceptor is a new formation brought about by immunisation, and, on the ground of the principle already admitted, it must appear probable that the amboceptor, like the antitoxins, possesses a marked monotropism for the corresponding antigen. It was therefore not the result of accident, but of logical sequence, that my first researches, carried out with Morgenroth, on the mechanism of hæmolysis led us to the fundamental conclusion that the amboceptor alone stands in direct relation to the cell, and is quantitatively anchored by it. This anchoring of the amboceptor takes place with a maximum of chemical energy—it occurs even at o° C.

The union of the amboceptor and the cell has no harmful results on the latter. On the other hand, the amboceptor-laden cell is exposed to the action of the complement, which by itself is harmless. As regards complements, what I have said about toxins holds true; they may be regarded as toxin-like substances which possess a haptophoric group, and a toxophoric group which I call the "zymotoxic" group. That

these groups are independent of each other, is well seen in the case of modified complement—"complementoid," as numerous test-tube experiments have proved. Of special interest are the conditions of their distribution. The real state of affairs, which has been thoroughly investigated, especially in the case of hæmolysins, is this: the intact erythrocytes do not unite with the complement, which, however, is anchored by the complex of erythrocyte and amboceptor. A closer acquaintance with the conditions which govern distribution in this matter, cannot be arrived at by the hypothesis that the erythrocyte is sensitised by the amboceptor in such manner that an action of the complement is rendered possible. If one accepts the theory of Bordet, which really consists of the denial of the existence of direct relations between amboceptor and complement, one enters the realm of pure speculation; for one must then presume new affinities between the erythrocyte and complement to arise under the influence of the amboceptor. For this assumption we have no grounds. Bordet's method of proof must, therefore, limit itself to indirect conclusions, and consists merely of objections to the view held by Morgenroth and myself,

that the amboceptor and the complement stand in direct relationship to one another. As a matter of fact, from all sides proofs of the existence of this direct relationship have been advanced, and I think that the great majority of my colleagues to-day accept my view, which is known as the amboceptor theory. It is true, as we have from the beginning insisted, that the distributive relation of the complement in the presence of the amboceptor is not that of maximum chemical affinity; indeed, we have, on the contrary, as a rule an exceedingly loose relationship which perhaps corresponds to a reversible reaction. To show that this relation is purposeful, we need only the following proof —amboceptors are already present in large quantity and of various kinds in the blood-serum of normal animals. What, then, would happen if the entire mass of normal amboceptors reacted with pronounced avidity with the complement? Obviously the entire mass of complement would be anchored by the complementophile groups of the amboceptors, and there would be no free complement present in the living body. The grave results of such a state of affairs are evident; as soon as the *necessity for* the action of complements with a

special kind of amboceptor arose, there would be no complement available, all having previously been used up for the action of indifferent amboceptors. It is thus owing to the fact that the complement is free or only loosely united to the amboceptors in the circulation, that at a given moment it is ready for use. The maximum stimulus to action is rendered possible by the anchoring of the amboceptor to the erythrocyte, the avidity of the former toward the complement being thus increased. This increase of avidity which consists in the chemical affinity of the complementophile group for the complement being carried to its maximum, represents the gist of our knowledge of the action of the amboceptor.

The amboceptor, therefore, exercises the important function of bringing about a specific modification of those conditions existing in the organism which determine the distribution of complements, and which otherwise are not very evident. It causes the complements to become monotropic by its union with the given substance. The complements are thus localised by amboceptors which have previously become united to the substance—cell or otherwise. At the same time this action represents a purposive

saving; if the complement were already a constituent part of the amboceptor, then—as the complement is easily destroyed—there would often be no action. The purposive saving is evident from the fact that only in case of need the amboceptor becomes able to combine with the complement, and from the fact that complementoids which, by reason of having lost the zymotoxic group, become incapable of action, have at the same time lost some of their avidity for the amboceptor. By this change in the distributive quality of the complement that is associated with the formation of complementoid, the sphere of action of the amboceptor is considerably extended. Of other possible influences which may govern distribution and action we have an indication given by the work of Ferrata. Under Morgenroth's direction he carried out a research, from which it appears that a complement is not a single body, one and indivisible; for in a salt-free medium it is split up into two components, which are capable of action only when they work in concert in a salt solution. As to the intimate relations of these two components, the researches which were carried out at the suggestion of Sachs by Dr. Brand appear *to indicate that in* the blood serum they are *always united.*

The increase of avidity, which forms the basis of the mode of action of the amboceptor, not only governs the phenomena which occur in the organism, but, since it lends itself to test-tube experiments, also opens up a wide field for serum diagnosis. If we remember that the amboceptors, by their union with the sensitive substances—cells or dissolved bodies—exert such a marked localising influence on the complements, it is evident that in a mixture of an amboceptor and its corresponding antigen, if the presence of the one constituent of the mixture be known, that of the other can be proved by the occurrence of the phenomenon of complement-fixation.

This method, which was elaborated by Neisser and Sachs on the basis of the work of Bordet, Gengou and Moreschi, for the medico-legal test of the source of blood by "complement deviation," depends solely on the principle of this increase of avidity. And, thanks to the genius of Wassermann, we possess a similar method of sero-diagnosis for some infectious diseases, the cause of which is at present unknown or invisible—a method which has yielded valuable results and gives great promise for the future.

In practice, too, we have a further advantage in the fact that the increase of avidity affects not one complement alone, but, as a rule, all the complements circulating in the blood. For the complement-deviation test we may then choose at will a complement which exercises any special function, and, merely on practical grounds, we choose hæmolytic complements. The peculiar power of the amboceptor to fix a large number of complements, is not to be wondered at in view of the biological function of the amboceptor. This I hold to be, under physiological conditions, that of seizing upon and elaborating nutritive substances. By its cytophile group the amboceptor is enabled to combine with substances of the most varied kinds, provided that they possess fitting receptors. We have, then, merely an increase of purposive function in the fact that the amboceptor is furnished with a host of complementophile groups which enable the most varied kinds of complement to act—it may be simultaneously. This is the consequence of the multiceptive nature of the amboceptor.

As regards increase of avidity, the fact that the anchoring of the amboceptor to the cell *causes the avidity* for the complement to be

increased, in no way precludes an increase of the avidity of the amboceptor for the cell taking place in consequence of the anchoring of the complement. Besides a few normal amboceptors of the blood-serum in which this is observed, we find that it occurs in a marked degree in the case of those poisons which act by the united action of two components, the part of the complement being played by lecithin. Thus, the power of forming lecithids has been studied by Kyes, who proved its existence in the case of snake and scorpion poisons, and by Morgenroth and Carpi, who studied bee poison. The action of the venom in these cases corresponds to that of an amboceptor, and the conditions for more careful analysis are very favourable; for snake poison and lecithin are both stable substances, and the latter belongs to a class whose chemical composition is known. Thus Kyes was enabled to isolate and analyse the substance produced by the reaction of snake poison with lecithin. Cobra-lecithid was found to be a mono-stearyl lecithin, and represents the result of a typical series of processes which certainly take place in cell life and in many phases of immunity. These processes occur in consequence of the union of albuminous substances, possessing special

province of the physiology of stimulation. Light, however, has been thrown on the subject of the specificity of reactions by the view I have expressed that the receptors of the cell-protoplasm are the seat of the process, and that their regeneration and elimination are the consequences of this. Supposing these processes to occur in the normal organism, and to be merely intensified in the case of active immunisation, it is possible to understand that antibodies of the most varied kinds may exist in the serum of the normal organism, and also to comprehend the processes of immunity by viewing them from the standpoint of the physiology of nutrition and metabolism. Thus I am glad to be able, by my conception of the problem, to come into entire agreement with Metchnikoff. The immense number of exceedingly various substances which possess haptine characters in the blood serum, substances of whose existence at one time one did not even dream, is thus to be explained as an expression of a many sided and differentiated action of the most varied organs. One may already by simple means differentiate the multitude of serum substances into antitoxins, amboceptors, agglutinins, precipitins, opsonins, *complements*, ferments, antiferments, etc., but a

deeper study of the subject shows that each of these divisions, in its turn, consists of a multitude of functionally different components, and thus a pluralistic view of the observed phenomena is the only justifiable one. Although in some quarters the endeavour is still made to reduce everything to the most simple form, I believe that such a rudimentary way of looking at things is not justified by the appreciably complex character of natural phenomena and vital processes; for we see, in the investigation of immunity, that when earlier opinions, based on experiment, must be replaced by newer ones, the process takes place always by the substitution of a complex conception in place of a simple one; I would remind you of Buchner's idea regarding alexin, which had to give way to the proved fact that all cytotoxins have a complex nature; and also I would remind you of the anti-complements, which we had supposed to be simple bodies, while it is now known that anti-complementary action is, as a rule, the result of the concerted action of two substances. In the case of the complements, too, in the light of Ferrata's researches, we must assume that the conception of these as simple substances is *wrong*. The more readily, then, may we

assume the existence of a multitude of substances as the cause of the various actions which are exerted by one and the same blood serum, a multitude whose existence has in numerous cases been proved. Objections which have been raised by several observers, especially by Bordet, against our method of proof, and which consist in stating that in every experiment the substance (whose unity is assumed) has been injuriously affected, and in attributing any difference to the varying degree of sensitiveness of the various test-substances alone, cannot be justified; for, if one takes the trouble to work—as I have always urged that one should do—quantitatively, such sources of error are immediately excluded from our conclusions. And even in spite of these, especially in the case of the proof of the multitude of complements, in many cases one has been able, by employing means of the most varied kind, to obtain either the loss of a certain function, or an absolutely disproportionate change of degree in isolated functions. In the plurality of haptines present in the serum we have a wide field open for more profound observation of the mechanism of receptor-metabolism, of the laws governing variations, and the influences which bring these about, a

field from which new light will be shed on human pathology and clinical medicine. Successful work in this field must proceed on a broad basis. One would have first to make exact observations regarding a large number of functions of human blood serum, and then one would have to investigate systematically in cases of all sorts of diseases, anomalies of nutrition, etc., the causes of departure from the normal, as to whether these result from the failure of certain functions, or from the existence of new functions acting under pathological conditions. Thus, without doubt, would be detected differences in the sum of the functions of the cell, and this section of the physiology and pathology of the blood might be named the blood canon.

I firmly believe that by extensive research we shall find that there exist great differences, the result of biological laws, and these will permit us to come to correct conclusions as to the origin of certain substances in the cell, and to apply these conclusions to diagnosis and therapeutics. Of course, the united action of many observers in many institutes, and the closest relations between clinical and laboratory work, are needful in order that progress may be made

in the direction indicated. The recent work of Wassermann, who by means of complement-fixation, was able to prove the existence in the blood serum of anti-bodies for certain nutritive substances (glycogen, albumoses, peptones, etc.), and to obtain an increased concentration of these by increased doses of the nutritive substances, appears to me to be very promising. In the case of pathogenetic or pathognomonic questions it does not appear to be of use to seek for those haptines which may be present in the blood as the result of immunisation; for in their case one would either find differences that are but slightly marked, or, if one found sufficiently-marked differences, one would have to be very careful in drawing conclusions from them. It would be better, therefore, to avoid using as test objects those substances which are already present in the normal body, or gain entrance to it by infection. In order to be able to draw absolutely correct conclusions as to the relations existing between certain substances in the serum and the normal or pathological activity of organs, one ought to choose cells or other elements, regarding which one may assume that they never come into *relation* with the human body in a natural way.

We have a small beginning in this direction, I think, in the research which I carried out with Wechsberg; we compared the behaviour of human blood-serum towards the trypanosomata in the case of healthy and diseased individuals, and we found that in cases of liver disease the amount of trypanosomicidal substances in the serum is markedly decreased, as will be seen from the subsequent table.

After Laveran had by his researches shown that the serum of man and of a few species of monkeys had a trypanosomicidal influence which was not possessed by the sera of other animals, it appeared worth while to pursue these researches further, and to study this action quantitatively, and when influenced by various conditions. For this purpose we employed a strain of the trypanosoma of *mal de caderas*, two or three control animals being also employed in each case, which succumbed at the latest on the fifth, and generally on the fourth day. The animals were injected with equal quantities in a similar manner, and as soon as the parasites appeared in the blood (on the second day) the curative serum was injected. By this means we obtained a titration of a number of human sera, regarding which I shall only give the

following particulars as to the results obtained with small doses.

As Laveran had shown, the normal serum caused the parasites to disappear, but, after a varying period, they again made their appearance and generally soon afterwards caused death. In cases in which $\frac{1}{8}$ to $\frac{1}{10}$ c.c. of serum was injected, the results in the majority of sera tested were extraordinarily constant, in the other cases death occurred between the tenth and fourteenth day.

The cases were as follows:—

Normal Serum I., death on the twelfth day.

Normal Serum II., death on the twelfth day.

Diabetes mellitus, death on the twelfth day.

Acromegaly, presumably between the tenth and eleventh day (0·0625 c.c. caused death on the ninth day).

Concretio cordis, death on the eleventh day.

Colica saturnina, death on the eleventh day.

Polycythæmia rubra, death on the twelfth day.

Carcinoma of stomach with metastases in the liver, death on the thirteenth day.

Peritoneal tuberculosis, death on the thirteenth day.

Leucæmia myelogenica, death on the fourteenth day.

In the cases of liver affections, on the other hand, the following results were obtained:—

(1) Carcinoma of the bile ducts with complete bile stasis: 0·5—1·0 c.c. serum, death on the fifth day; 2·0 c.c. serum, death on the eleventh day.

(2) Carcinoma of the stomach, with jaundice, 0·1—0·25 c.c. serum, death on the fifth day.

(3) Alcoholic cirrhosis of the liver, 0·125 c.c. serum, death on the sixth day.

(4) Biliary cirrhosis with jaundice, 0·125 c.c. serum, death on the seventh day.

(5) Alcoholic cirrhosis of the liver, 0·125 c.c. serum, death on the eighth day.

(6) Biliary cirrhosis with jaundice, 0·125 c.c. serum, death on the thirteenth day.

Thus there was observed in every instance, with the exception of case 6, a distinct, though varying, deficiency in the trypanosomicidal properties of the serum, and that this deficiency was not merely an apparent one, produced by the presence of inhibiting substances, *e.g.*, the products of bile stasis, was shown by experiments in which a mixture of equal parts of the serum from case 1 and from the acromegaly case were injected: 0·5 c.c. of the mixture,

death on the sixteenth day; 0·25 c.c. of the mixture, death on the tenth day.

Naturally one can only expect to find such marked differences in the case of those substances which owe their origin to a certain organ or combination of organs. If the place of origin of other haptines is widely diffused throughout the connective tissue of the organism, one can scarcely hope in such a case to achieve much of value for diagnostic purposes. An extensive organisation, which does not confine itself to investigation of a single haptine-substance, or indeed to a few such, is therefore necessary, in order that more exact analyses may be made, and results of a practical value be obtained.

Looking back upon what I have said, you will see that it is the principle of distribution which governs the processes resulting in active immunisation. The antibodies—the protective substances in the serum—all possess the power of reacting, with maximum chemical energy, with their corresponding antigen, as, *e.g.*, in the anchoring of bacterial cells. This anchoring is a necessary preliminary for further reaction, which may be of the most varied kind. *We are* acquainted with a number of such

haptines or antibodies, of which the following are examples:—

(1) The agglutinins, which cause clumping of the cells.

(2) The amboceptors, which play the part of carriers in the action of the complement.

(3) The opsonins, which render bacteria liable to be seized by the phagocytes; also those haptines which are directed against the contents, or metabolic products, of bacteria.

(4) The antitoxins.

(5) The anti-endotoxins, which are directed against the endotoxins, to our knowledge of which Macfadyen, whose early death the scientific world deplores, has contributed so much.

(6) The precipitins.

(7) The antiferments, which are directed against certain ferments of the bacterial cells, *e.g.*, pyocyanase.

There is no doubt, however, that many more haptines exist. In order to obtain an idea of the extreme diversity of the phenomena which cause immunity, one must look for other substances whose functions are those which characterise the haptines—the substances, for example, which prevent cell division, or those which combat the biological adaptation of

bacteria. The possibilities which I have here indicated appear to be limitless, and the need for study in this direction is evident.

I would here express my dissent from a prejudice which often makes itself felt, to the effect that in this matter there exists a profound contradiction between humoral and cellular immunity. As a matter of fact, to assume that the action of antibodies is merely a process of humoral pathology, is to put an artificial construction on the facts observed: for the side-chain theory is founded upon the view that the antibodies are purely and simply the product of cellular secretion, and that with their appearance in the blood there are associated changes in the cells which correspond to the phenomenon of serum immunity. That the action of antibodies takes place in the juices of the organism is an incontestable fact, which, however, in view of the cellular processes which give rise to it, cannot with justice be claimed to be evidence in proof of the correctness of humoral pathology, otherwise we must consider the action of ferments to be one of humoral physiology.

In the Protean forms of the phenomena of immunity, of course, the action of haptines by no

means excludes phagocytosis; destruction of the bacteria outside the cells and their assimilation by the phagocytes are processes which may take place alongside each other, and, by their simultaneous action, increase the protective power. A special proof of the importance of the study of haptines appears to me to be the fact that—as the opsonin theory, which we owe to Sir Almroth Wright, has made more evident, specific haptine reactions form the basis also of phagocytosis, which Metchnikoff has studied in so masterly a manner. The opsonins and cytotropic substances render the bacteria liable to attack by the phagocytes, and here we have a field in which humoral and cellular processes meet. One cannot, however, say that the possible causes of immunity are confined to haptine action and phagocytosis. Perhaps the atreptic view, by which differences of degree in avidity on the one side or on the other are presumed, is correct in many cases in which other influences are at work. This view of the case I shall treat more fully when I come to speak of carcinoma.

The immunity of an animal is, therefore, explained as being due to the great energy of the cells of its body, which are able to appropriate

nutritious substances for themselves, and in so doing to deprive parasites of them. The opposite condition must be due to a certain disposing influence, and immunity of the parasites must be a condition of the cause of infectivity. The bacterial cells may in the same way be immune against haptine substances, and may withstand the action of the serum.

Thus there exist unstable relations between immunity and infection, and between parasite and host, relations which may depend on the most varying influences, and which lead up to the phenomena of reversible action, which calls for further study.

One cannot, therefore, go to work in a one-sided way when analysing and judging the various forms of phenomena, but must carefully consider together all the factors in question. The study of every possibility will bear fruit and make for an understanding of the processes of infection and immunity. I believe, however, that I have shown the influence exerted by the haptines upon the cause of infection is of great importance, not only when these are viewed as destroyers of the cause of infection outside the cells, but also when viewed in *connection* with the results of their anchoring

power, chief among which at present stands phagocytosis.

It is our task to advance by a more accurate and more extensive study of *all* the haptines and their actions, and in the first place we must gain a knowledge of the influences exerted upon the causes of infection by the distribution and action of dissolved substances whose action is cytotropic, so that we may obtain a nearer insight into the manifold secondary phenomena which arise from them.

LECTURE II.

On the Atreptic Function.

In my first lecture I dealt with the anchoring phenomena exhibited by the different kinds of parasitotropic substances, which thus aid the body in the process of recovery, and I chiefly discussed, in their historical sequence, the three therapeutically important agents, the antitoxins, the bacteriolysins and the opsonins of Wright. I, however indicated, that with these substances the possibilities were not yet exhausted. To-day I shall speak of a different series of processes which may aid the organism to fight its adversaries, processes of a more passive nature, in which a secretion of active attacking substances does not take place.

You will remember that in the early days of immunity the exhaustion theory played an important *rôle*. Pasteur thought that when the body overcomes an infection, there are *removed from* it certain substances necessary

for the development of that particular bacterial species; should the same kind of bacterium again penetrate into an organism thus modified, it would not find those substances necessary for its growth, and thus a new infection could not occur. This theory has of late years, and especially since the discovery of the different kinds of antibodies, ceased to be of importance in practical medicine. In point of fact, it is hard to imagine that in the living body, as a result of some infective disease, which need not have produced any serious disturbances, there should have occurred a complete and permanent disappearance of certain chemical substances. That during disease the internal secretions should become disturbed, and that possibly at this time certain cellular products should be temporarily removed by excessive consumption and insufficient nutrition, is possible and even probable, but it is quite unintelligible how, after complete recovery, such a state of things could still persist. Yet this old theory seems to me to contain a nucleus of truth, as is so often the case. The chief point of Pasteur's theory is that in the modified body the bacteria should not be able to assimilate certain food substances. Now, Pasteur's idea

is that this could only take place if the substances in question were absent; this is obviously incorrect. But this hypothesis is not necessary in order to explain such insufficient nutrition of the bacteria; it suffices to assume that those substances may still be present, but that the parasitic agents in question are incapable of absorbing them; in other words, that the substances have ceased to be at the disposal of the bacteria.

This phenomenon, at the time of my cancer studies, I embodied in the definition of "Atrepsy." I will give you a few examples from my researches which will make clear what I mean by this definition, and I will begin with a fairly simple case, viz., that of the cobra venom.

This poison produces in the body a number of very different injuries, *e.g.*, it affects the nervous centres, the subcutaneous tissue, the red blood corpuscles, the endothelia, etc. But whilst the manifold pathological conditions produced in the body, *e.g.*, by corrosive sublimate or any other well-defined chemical substance, are in every case the effect of one substance on different organs—as, for *instance,* nephritis, inflammation of the salivary

glands and necrosis of the intestinal mucosa are the result of the *one* poison, corrosive sublimate,—the case is entirely different with snake venom, for there each separate and individual effect is the result of a different individual poison. Thus, one can prove with absolute certainty that the effect on the nervous tissues is produced by the neurotoxin, that on the blood corpuscles by a hæmolysin, and the inflammatory changes by a special endotheliotoxin. It has been shown that those components of snake venom which attack the red blood corpuscles are present in such a form that by themselves they do not suffice to destroy them.

By following up the researches of Flexner and Calmette, my former assistant and friend, Dr. Kyes, has succeeded in throwing some light on these difficult subjects. He proved that the hæmolytic component of the cobra venom is an amboceptor, which, however, differs from the bacteriolytic amboceptors in not combining with the complements of the blood, but which enters into union with a chemically well-defined substance, lecithin.

By shaking cobra venom solutions with a solution of lecithin in chloroform, one can show that the whole hæmolytic toxin passes quanti-

tatively into the chloroform, whilst the watery portion has retained the whole of the true poisonous principle, viz., the neurotoxin. From the chloroform solution one can isolate the active hæmolytic principle by precipitation with ether; it can thus be obtained in the form of a white powder, which, on chemical analysis, gives figures which almost exactly fit the formula of a mono-stearyl-lecithide. In this process there has, therefore, been split off one of the two molecules of fatty acid; this can be recovered quantitatively from the ether.

The substance thus obtained is produced by the union of a small quantity of the hæmolytically active part of cobra venom with a relatively large number of lecithin molecules. It is easy to prove that in this we really have to do with a chemical compound, and not the result of an absorption similar to that recently described by Michaelis between rennet and mastix; for it is impossible to split up the substance by solvents into two components, viz., mono-stearyl-lecithin and the cobra venom amboceptor, which would be possible if it were only such a mixture. On the other hand, in conformity with its altered chemical character, *the substance* has obtained new properties not

found in cobra venom. For, in the first place, it is distinguished from the cobra amboceptor by having become so completely thermostable, that solutions of this cobra-lecithide can be boiled for hours without losing a fraction of their efficiency, whilst under similar conditions the original venom would be rapidly destroyed. A further important property is shown by the fact that cobra-lecithide, even in small quantity, instantaneously and without any incubation period dissolves the erythrocytes, whilst snake venom, even in the presence of lecithin, only produces a similar effect after several hours. The long incubation period observed in experiments with the native poison is explained by the fact that the real poisonous body has first to be formed by a synthetic process, which, in dilute solutions and at ordinary temperatures, may naturally require some time for its completion. As you see, I believe with Kyes, that the hæmolysis of the red blood corpuscles is the result of a similar lecithide formation.

It is interesting to note the action of cobra venom towards different kinds of red blood corpuscles. It has been found that the corpuscles of the guinea-pig, man and rabbit, are at once dissolved by solutions of the poison, but that

this is not the case with sheep or ox corpuscles. These latter, however, are also dissolved if a small amount of lecithin is added to the mixture.

How is one to explain the fact that cobra venom cannot by itself dissolve certain kinds of blood?

It has been shown that in all bloods lecithin is present in fairly large quantities. If in spite of that only certain kinds of blood are affected by cobra venom, this must be explained by the assumption that in every blood lecithin is not present in the free state, but that it is combined with certain substances of the erythrocyte's stroma, and that the firmness of this union differs considerably in the different kinds of blood. That even in the resistant erythrocytes —*e.g.*, those of the sheep—lecithin is present as the activating substance, can easily be proved by breaking down the compound of proteid and lecithin. This can be done by extracting with alcohol. The alcoholic extract of sheep's blood corpuscles can be shown to activate a mixture of snake venom and sheep's corpuscles.

The results are different if, instead of cobra venom, other poisons are employed. Thus, the *poison of Bothrops lanceolatus* is absolutely unable

to dissolve any of the above-mentioned bloods without the addition of lecithin, whilst the poison of *Trimesurus anamalensis* dissolves only the corpuscles of the guinea-pig, that of *Bungarus fasciatus* only the corpuscles of man and guinea-pigs, but not others. From these researches we may deduce two facts, viz.: (1) That the hæmolytic amboceptors of snake poisons possess different affinities for lecithin, this affinity being lowest in bothrops venom, which dissolves none of the before-mentioned corpuscles, and highest in cobra venom, which dissolves three of the five kinds; (2) that the firmness of union of the lecithin present in the different blood species varies, being highest in the sheep and the ox, lower in the rabbit, still lower in man, and lowest of all in the guinea-pig. You see here very clearly one of the conditions which are of importance for the definition of atrepsy, namely, that of indisposability. Lecithin has an affinity for certain constituents of the cell and it also has an affinity for the constituents of the poison; the difference between these two affinities decides whether cobra lecithide can be formed and hæmolysis can thus be produced. If the affinity of the constituents of the stromata is higher, hæmolysis does not take place, i.e., the

lecithin is not at the disposal of the cobra amboceptor.

I will now discuss a further similar case, namely, that of certain phenomena observed by me in the course of my studies on trypanosomes. As some of you may know, I have, together with my assistants, Dr. Röhl and Dr. Browning, proved that trypanosomes can be rendered resistant towards all those agents which are employed for curative purposes. If, *e.g.*, a mouse infected with trypanosomes is treated with fuchsin, the parasites disappear, but return after a longer or shorter interval. If the treatment is then repeated, a similar result is observed. Thus this alternation of relapse and treatment can be repeated several times, though not indefinitely, for in course of time the effect of the fuchsin becomes more and more unsatisfactory and finally disappears altogether. If now the trypanosomes are transferred from such an animal to normal mice, these parasites are now, even in the normal mouse, found to be no longer capable of being influenced by fuchsin. There has thus been formed a fuchsin-resistant or "fuchsin-fast" strain. I have chosen this expression because I found that such a resis*tance, once acquired*, appears to remain un-

altered even after as many as a hundred successive passages. We have up to now produced similar strains resistant to arsenical preparations, to trypan-red and trypan-blue as well as to fuchsin. In my next lecture I shall enter more fully into the importance of these strains.

I will here discuss one point only which is connected with to-day's subject, viz., the question as to the *origin* of the resistant strains. I have made the interesting observation on an arsenic-fast strain which was obtained by several years' treatment with atoxyl, and which was then made still more resistant by means of a very active arsenical preparation (No. 379), which I shall briefly call "Trypocid." This strain is distinguished from normal trypanosomes in that it is no longer influenced by this highly active arsenical preparation. One would have expected that also in test-tube experiments, it would have shown a high resistance towards trypocid. Of course, such an examination could only be carried out by comparing it with other strains of trypanosomes. For obvious reasons I employed the original strain from which this resistant one had been derived, and which had been cultivated in the laboratory. I chose two animals containing about the same number of

parasites and mixed their bloods with solutions of trypocid of different degrees of concentration. I now observed the very unexpected phenomenon that in these mixtures the immune strain was far less resistant to higher doses of trypocid than the normal one. Thus whilst concentrations of 1 in 500 to 1 in 1,000 almost instantaneously killed the immune strain, the control strain retained its motility unimpaired in these concentrations for not less than five minutes.

We have here, therefore, one of those striking phenomena which have already been met with in the study of immunity and which consist in the simultaneous occurrence of immunity and hypersensibility in one and the same organism; but the case here is somewhat more simple, since we are working with unicellular organisms, and may therefore hope to obtain a more accurate idea of the case.

Allow me at this juncture to suggest to you a few fundamental principles. In order that a given poison, *e.g.*, the arsenical preparation, may act upon the trypanosomes, it must contain certain chemical groups which seize upon the latter. These groups I will distinguish from the ordinary receptors, which play an important part in the theory of immunity, by the name of

"chemo-receptors." In like manner the bodies of the higher animals must possess such chemo-receptors in certain organs, as they are also injured by the poison. If now a mouse infected with trypanosomes is injected with the arsenical medicament, this substance will be distributed between the parasites and the organism of the mouse. If the receptivity of the parasites is the stronger, they will be killed in the organism; in the alternative case, they will not be destroyed. Consequently the curative result obtained when experimenting under normal circumstances represents also the differential between two avidities; *i.e.*, if a mouse infected with the normal strain of trypanosomes is injected with the arsenical preparation, the "trypanotropic" force of the drug is stronger than the "organotropic" force; we therefore obtain a curative effect with doses which are not injurious to the organism of the mouse.

In the resistant strains this is not the case, since the same doses show no trace of action on the parasites. One might imagine that this result was due to the trypanosomes having lost their chemo-receptors: but it is not necessary to make this assumption, and Nature has in its *wonderfully* complex mechanism found another

and far more economical expedient than the loss of a perhaps necessary chemical structure. It is quite evident that the same result could be obtained if the chemo-receptors of the trypanosomes were so far reduced in their affinity that the proportion of distribution was altered in favour of the chemo-receptors of the organism. That, indeed, is what takes place. Had the chemo-receptors quite disappeared, the immune strains would also in the test-tube experiment be more resistant than the normal ones; as a matter of fact, with certain preparations the contrary occurs.* The phenomenon is most easily explained by the supposition that the affinity of the chemo-receptors has become adjusted in such manner to the counterbalancing affinity of the mouse organism, that in the mouse no more arsenic remains at the disposal of the

* The fact that these resistant strains are, in the test-tube experiment, affected even more severely than the normal ones, is the result of the co-existence of resistance and hyper-sensitivity in the parasite's protoplasm. Such a combination of immunity and hyper-sensitivity has repeatedly been observed in the course of the immunisation of higher animals, and this is now assuming increasing importance. It is the more interesting to note that the same phenomenon occurs also in uni-cellular organisms. The explanation of this observation will be given later.

trypanosomes. If, however, this counterbalancing effect of the mouse's organism is removed, the poisonous agent can with full force seize the trypanosomes. I would even go so far as to say that we have to do in this case with a biological adjustment of the greatest delicacy, and that Nature limits herself to the most strictly necessary gradations and never allows a reduction of the affinity to its minimum.

As a proof of this I may mention a similar biological observation. I sent my resistant strain to the Liverpool School of Tropical Medicine, where it was found that the atoxyl-resistant strain showed the resistance as described by me only in mice but not in rats. This result was, of course, very surprising, but you will easily understand it on the basis of the previous results, by the supposition that the avidity of the rat's organism for trypocid is inferior to that of the mouse. In the rat, therefore, the trypanocidal effect is preponderant, since the trypanosomes are still able to seize upon the poison.

Let me now pass on to a very different field of research. I should be guilty of carrying owls to Athens, if I were here to speak in detail of the nature and importance of Jenner's great

discovery, which represents an epoch in our campaign against disease and the beginning of the era of immunisation. I shall therefore enter only into one question, which throws a little light upon that modification of the variola virus which is produced by its passage through the cow. As you know, animals are also affected with small-pox-like diseases which are closely related to human variola. Such diseases are the so-called "sheep-pox" and "bird-pox." The latter affection has recently been studied at my Institute by Dr. Sticker and Staff-Surgeon Marx. It is easily transferable by cutaneous inoculation to fowls and pigeons, and produces extensive small-pox-like hypertrophy of the epithelium. In the diseased epithelial cells highly staining spherical cell-inclusions are found, which must be identified with the corpuscles of Guarnieri that are found in epithelial cells infected with small-pox or vaccine. Animals which have passed through the disease have thus acquired a complete immunity against subsequent infections.

As was shown by my assistants, the virus of bird-pox can be filtered through filter-candles. It can be transferred to fowls and pigeons. In fowls the virus retains its pathogenic properties

unaltered for any number of inoculations. But the results are very different if one tries to inoculate with the diseased products occurring in pigeons. For although the inoculations can also be carried out in pigeons for any number of times, yet in such series the virulence for fowls has completely disappeared. You see, therefore, that we are here dealing with a process closely related to the modifications which the variola virus suffers in the organism of the cow. The only difference exists in the fact that in the one case the virulence for the original host is only reduced, whilst in the case of bird-pox it is completely destroyed. In my view this can be explained only in one way, for it is obviously impossible to suppose that by *one* passage through the pigeon's organism the parasites in question should obtain a new set of receptors capable of seizing upon antagonistic bodies present in the normal fowl's serum. It is far more natural to suppose that we have to do here with a form of atrepsy. It is safe to assume that the chemical composition of fowls' and pigeons' tissues is not identical, and that therefore the parasites, in their passage through the pigeon, must assimilate substances different to those assimilated in their passage through

fowls. Therefore, that part of the receptors which deals with the nutritive substances in the fowl's organism is not in use during the passage through the pigeon. In view of their great instability it is possible that this portion of the receptors may become atrophied, whilst the receptors specific for pigeon's substances become correspondingly increased. Thus the micro-organism would lose its power of assimilating certain substances of the fowl's organism. If such a parasite were transferred back to the fowl—supposing one of the specific constituents of fowls to be necessary for its proliferation—it would no more be able to grow in the fowl.

This is, therefore, a case of the loss of certain receptors which are absolutely necessary for nutrition. That such a change, having been once acquired, should be permanently transmitted from one generation to another, is hardly surprising in view of my recent observations on the modifications of arsenic-resistant trypanosomata. Obviously, the total loss of the chemical structures in question must render such modifications irreparable. Such characteristic instances as those in which a single passage produces a permanent loss of virulence for a certain animal species, are not very frequent.

Correspondingly we may presume, that the variola virus, when passing through the cow, permanently loses certain atom-groups which cannot be regained; I believe, however, that in cow-pox this loss is of a lesser degree than in bird-pox.

On the other hand, in bacteriology we frequently find that such alterations produced by certain animal passages are not of a permanent nature. Thus one can, by successively passing streptococci through animals of one species, obtain a maximum of virulence for that species. It is possible, therefore, at will to produce a rabbit-strain, a mouse-strain, etc. But one can, by repeatedly passing such a strain through animals of a different species, transform it into a different strain, and thus in course of time obtain from a rabbit-strain a mouse-strain. This is therefore a case of atrepsy of the receptoral apparatus of the bacteria for the animal species in question, but this atrepsy differs from that of the variations of small-pox by not possessing a permanent character.

Probably the majority of so-called non-pathogenic micro-organisms, if introduced into an animal's body, perish by this mechanism. It is not neccessary to assume the presence of special

poisons in the body, it suffices to suppose that the bacteria in question do not find the needful means of existence in the body and therefore cannot multiply. This being the case, they cannot for any length of time remain alive in the body, for then the latter's defensive forces, its phagocytes, come into action and destroy the invaders in a non-specific manner.

In the case of the pathogenic micro-organisms it will, however, as a general rule be safer not to attribute too great an importance to atrepsy. But it is evident that micro-organisms can only be pathogenic for a certain animal if they find in it possibilities of nutrition. Yet, to my mind, quite a number of infections are characterised by the fact that the micro-organism, with the exception of only a few remnants, becomes atreptic. As an example of such an occurrence I would mention the fine researches of my friend, A. Neisser, who found that monkeys injected subcutaneously or intra-peritoneally with great quantities of syphilitic virus became neither infected nor immunised.

It does not appear possible to assume that in the serum of monkeys, substances should be present which destroy the causative agents of

syphilis. It will be more correct to imagine that the delicate spirochætæ have lost their power of infecting because they have found no suitable nutritive bodies in the serum.

On the other hand we know, from the fundamental experiments of Metchnikoff and Roux, that typical primary lesions can in apes be obtained in any part of the skin, in the lower monkeys chiefly in the eyebrows and penis. Infection could also be produced by rubbing diseased tissue on to the cut surface of the testicle. On examining the inner organs of the monkeys, Neisser found the virus to be present only in a few organs, especially the hæmatopoietic organs, viz., the spleen, the bone marrow and the lymphatic glands, as well as in the testicles, whilst all the other organs proved sterile. For this reason Neisser thought that the primary processes in certain defined tissues were of importance for the occurrence of the generalised disease. If we follow this view, it will be most easy to explain the state of affairs by the hypothesis that the serum of monkeys does not contain properties sufficient for the nutrition of the spirochætæ, whilst in certain cells of these animals, viz., the epithelia, the testicular cells and the leucogenic cells, sub-

stances permitting proliferation of the spirochætæ must be present. Probably there exists only a minimum of such substances, auxiliary, but indispensable for their nutrition.

I may here remind you of the fact that the influenza bacillus, as was shown by R. Pfeiffer, is not able to proliferate in ordinary culture media, but that a trace of hæmoglobin is necessary for its growth. In syphilis I imagine the case to be somewhat similar. All the organs which can be primarily infected with syphilitic virus would thus be the carriers of this specific auxiliary substance.

The case is more evident, macroscopically, in a great number of very different affections, *e.g.*, variola and vaccinia, bird's and sheep's pox, foot- and mouth-disease, trachoma, rabies, fowl's pest and scarlatina. In all these infections there are found in the epithelial cells the above-mentioned peculiar cell-inclusions, which in small-pox are called Guarnieri's corpuscles, in rabies Negri's corpuscles. According to recent investigations we are justified in supposing that these inclusions do not themselves represent the parasites, but that they are derived from the cells and consist, according to von Prowazek, of *plastin and* nuclease. Within these masses

there would lie enclosed the exceedingly minute disease-producing agents, these agents being in general so small as hardly to be accurately recognisable by the microscope, with the exception of the inclusions in trachoma, where the parasites are clearly visible and bear the stamp of something organised. We may safely assume that in all these cases the specific localisation of the parasites in certain distinct kinds of cells, points to the fact that in these cells, notably the epithelium cells in small-pox and trachoma, and the ganglion cells in rabies and fowl pest, such specific auxiliary bodies for the parasites are present, whilst in all the remaining parts the micro-organisms would encounter conditions of atrepsy.

The atreptic immunity, which results as I stated from certain substances not being at the disposal of the parasites, seems to me to play an important *rôle* in the study of tumours.

I may even say, that I first formed the idea of atrepsy as the result of numerous experimental observations upon malignant mouse tumours which are of cardinal interest for modern cancer research. I believe that a number of clinical and experimental facts in this sphere of knowledge can only be explained by this hypothesis.

As you know, mouse cancers are tumours showing a far-reaching analogy to human cancer, and it would therefore appear most justifiable to regard the experiences gained with them as the solid foundation of an experimental study of tumours. Besides several analogies, however, the mouse tumours show certain special features, chief among which is the mode of formation of metastases. It is most remarkable that the highly virulent carcinomata which have been cultivated in numerous generations, very rarely show macroscopically visible metastases, whilst the slowly growing spontaneous tumours relatively more often, though also not very commonly, form large secondary nodules in the lungs. Our interest in this peculiar behaviour must even be increased, since Haaland has shown that microscopical metastases are by no means uncommon, but that they almost always remain below the limit of macroscopical visibility. I endeavoured to solve this problem by making, so to speak, artificial metastases, viz., by studying in animals affected with an inoculated tumour the result of a second inoculation. I found the interesting fact, that the effect of the second inoculation was inversely proportional to the energy of growth of the primary

tumour. In rapidly growing sarcomata and carcinomata the second inoculation almost always had a negative result; at best, the second tumour remained far inferior in size to the primary one. Only in the very slowly growing chondromata was there no distinct difference in their energy of proliferation. These results agree with the following observation, which has been confirmed thousands of times. If, as is regularly done in my Institute, the inoculation is carried out by introducing the capillary tube at the groin and pushing it up as far as the axilla, the greater quantity of inoculated material will of course be deposited at the latter site, whilst only a small portion will remain at the groin. In the case of the rapidly growing carcinomata and sarcomata the large principal tumours almost always develop in the axilla, whilst a minute nodule is usually found in the groin. In the case of the chondromata, however, both tumours usually show a fairly equal development. Thus, in the latter case, the macroscopical appearance somewhat resembles that of an hour-glass, whilst in the former it is more like a balloon with its car attached.

To my mind, the explanation of this pheno-

menon is as follows: every proliferation depends in the first place on the avidity of the cells for the nutritive substances. Normally, there are certain well-defined laws of distribution, which guarantee the proper working of the organic functions. The avidity of the tumour cells is increased, as compared with that of the body cells. The more energetically a tumour proliferates, the more powerfully does it attract the nutrient substances from the blood. In the case of a rapidly growing tumour it may therefore very easily occur, that for such cells which are under very unfavourable conditions of nutrition, *e.g.*, cells inoculated and metastatically carried away, there is an insufficient supply of nutrient substances, and that they therefore either perish from atrepsy or at least are unfavourably influenced in their growth. From this point of view it is also quite evident that slowly growing tumours can attain far greater dimensions than rapidly growing ones, since in the former the consumption of nutrient matter, in spite of their size, is less than in the latter, and thus the entire organism is injured to a lesser extent.*

* In connection with this, attention may be called to the recent observations of Haaland *(Berl. Klin. Wochenschr.*,

I fail to understand how von Dungern can think himself justified in doubting the correctness of this explanation, on the ground that hitherto the experimental proof of a permanent increase of cellular avidity by increased nutrition has not been adduced. I have never asserted that we can by artificial means permanently raise the cellular avidity. Such a power would obviously involve the possibility of artificial tumour production. There is therefore no contradiction, as von Dungern imagines, between my views and the facts that by artificial hyperæmia better nutrition is obtained, and that, after removal of large parts of the body, *e.g.*, amputation at the thigh, the hypernutrition of the individual is evidenced, not by the proliferation of functionating epithelial cells, but by increase of the adipose tissue. From the fact that the increased avidity causes an increased attraction of nutritive material, it does not follow inversely that copious nutrition must increase the avidity. An indirectly injurious effect of more highly avid cells on less avid ones can therefore only become evident, if the body does not

1907, No. 23, p. 718), which show that pregnancy very often produces a retarding influence on the growth of such tumours.

possess sufficient nutrient matter to saturate every avidity. The origin of the higher avidity of the tumour cells is quite unknown to us; for this question means neither more nor less than the last problem of tumour ætiology.

An entirely different significance must be ascribed to the specific growth-stimulating bodies or "hormones," for the existence of which we have received irrefutable proof chiefly by the work of Starling. As you may know, he observed lactation in non-pregnant females as the result of injecting emulsions of embryos. But, of course, I should never think of asserting, as one might be led to think by von Dungern's explanation of my views, that as the result of over-nutrition a typical lactation might be set up in a virgin.

Such specific growth-stimulating bodies play an important *rôle* also in other cases, and are intimately connected with atreptic immunity. Thus we know that the influenza bacillus cannot do without hæmoglobin for its growth. Therefore it is quite easy to cultivate the bacillus directly from the sputum, which almost always contains small quantities of hæmoglobin. But if we do not artificially add hæmoglobin to the *sub-cultures*, the bacteria very soon die in them.

Specific auxiliary growth-substances we must also assume in all those infective diseases which are characterised by a marked localisation of the poison, as in bird-pox and syphilis.

The case is quite similar in that form of atreptic immunity which follows from my experiments of inoculating rats with cells of mouse tumours. As you know, hitherto no one has ever succeeded in transferring normal or tumour tissue to animals of a different species. The limits of transplantability coincide with those of bastardisation. The question, however, now arose, as to whether the same barriers held good also for our highly virulent tumour material. To that end I employed the rat as the animal phylogenetically most nearly related to the mouse. In fact, if a virulent mouse tumour is inoculated into a rat, the result is quite different from all hitherto known true cases of transference of tissue outside a species; for during the first eight days the cells, both of carcinomata, sarcomata and chondromata, proliferate in the rat just as they do in the mouse. During this time tumours arise from the size of an almond to that of a date, containing numerous mitoses and microscopically differing in no way from the mouse tumours. After that time,

however, the limit of their growth is reached and there now follows gradual resorption, which is concluded after another week or two. If the tumour at its maximum development is transferred to another rat, it does not gain any foothold there, whilst if it is re-inoculated into a mouse it again proliferates luxuriantly. It is possible to keep on carrying out this zig-zag inoculation from mouse to rat and from rat to mouse, and again from mouse to rat, etc., for any length of time without the slightest check to the energy of proliferation being encountered. The question now arises, how it is possible to explain, on the basis of these facts, the immunity of the rat towards mouse-tumour cells.

We can at once exclude the existence of a natural immunity by antibodies in the rat, in view of the marked initial proliferation of the tumour. On the other hand it would be possible to imagine that the resorption of the tumour might be the result of an active immunisation of the rat. That such immunisation ultimately occurs may be proved by the fact that the result of re-inoculating a rat, in which a first tumour has been absorbed, with a second tumour, is always negative.

I do not, however, consider it permissible to

identify these two manifestations of immunity. For in the first place it is highly improbable that, during the period of rapid proliferation of the tumour in the rat's organism, any considerable absorption of tissue elements should occur. Further, previous to that period, a production of antibodies has not been proved. Lastly, since the tumour, when re-inoculated into the mouse, never shows ever so slight a reduction of virulence, one would have to suppose with von Dungern that the anti-substance formed in the rat's organism was activated only by the complements of rats but not of mice. This, again, has up to the present not been proved.

A far simpler and more natural explanation of all these phenomena is afforded by my hypothesis of atrepsy. According to this, the mouse-tumour cells require for their growth not only the ordinary nutritive substances which the rat can also supply to them in ample quantity, but, besides that, some well-defined substance which is present only in the mouse's organism.

In the rat, then, these cells would only be able to go on growing so long as there still remained with them some of the specific growth-stimulating substance that was introduced with them at the time of inoculation. When it has

all been used up, growth can only be further stimulated by giving a fresh supply of this substance, *e.g.*, by re-inoculation into the mouse.

Just as it is impossible to explain this immunity of rats by antibodies, one cannot thus explain that form of immunity in mice which is evidenced in the number of positive results following inoculation with different tumour strains. Generally speaking, the proportion of positive results for one strain, with the exception of unavoidable chances, varies only within narrow limits. Thus, the strain of carcinoma which we cultivate in grey mice regularly grows in about 20 to 25 per cent.; Jensen's tumour, according to the publications, in about 40 to 60 per cent., our other carcinomata and sarcomata in 90 to 100 per cent., and our chondroma unexceptionally in 100 per cent. The constancy of these differences can never be explained by the assumption of antibodies, but only as the expression of a certain vitality of the tumour cells which is constant for each strain.

The atreptic condition is most clearly shown in the cases repeatedly mentioned by Michaelis, Bashford and Haaland, where the transference to other races of mice of such virulent tumours was either quite impossible, or was only successful

in a very low percentage of cases. In the latter a gradual adaptation to the strange culture medium could only with great difficulty be obtained.

From my previous remarks it follows that the increased avidity of the cells for the food substances is the most important characteristic of the tumour cells. But this increase does not suffice to explain all the phenomena observed. Albrecht already insisted that for malignant tumours one must admit not only an increase but an alteration in the assimilation, in such manner that the "structure materials" taken up from the surrounding media must in some way be bound or laid up "until they had reached an amount sufficient for the division of the cell." Besides this increased food-absorption, the result of action of the receptors, chemiotactic remote efforts must, to my mind, play an important *rôle*.

Such a phenomenon is very well shown by the chondroma which has been cultivated for many years in my Institute. This tumour shows a well-marked chemiotactic effect on the blood-vessels, in such manner that even small inoculated tumours, a week or two old, shine blue through the skin, whilst larger ones in their

entirety present the appearance of a hæmorrhagic tumour. This angiotactic attraction of the blood-vessels is an essential condition for the growth of the tumours; for if, as is the case in intra-peritoneal inoculation, these angiotactic properties cannot become active, or if they have been lost, as is the case in immunised animals, proliferation remains very limited both as regards its duration and its extent, and it rapidly ceases whilst the whole tissue becomes necrotic.

As you see, I have dealt with a number of apparently very different subjects of biology and pathology, which are, however, united by a common bond. We have before us the competition of the different individualities for their nutrient materials. The majority of such chemical compounds are organically bound within the cell to its constituents. This is, I think, especially true of the lipoid bodies, like fat, lecithin and others. In this respect I may, perhaps, remind you that normal tissue, *e.g.*, the cortex of the kidney, contains fat in such a hidden modification, since it shows fat granules neither microscopically nor by the osmic acid reaction. If one examines a similar organ in *the stage of* fatty degeneration, it shows macro-

scopically a white colour, and by microscopical examination with osmic acid shows an enormous number of fat granules. Yet, by extracting a normal and such a diseased kidney with ether and by examining the ether residue, Dunham showed that the quantities of extract thus obtained, which is but a mixture of fats and lecithin, is exactly the same in both cases. In this case, therefore, the occurrence of fatty degeneration consists only in the fact that the organic union between the proteids and the lipoids has ceased, and that the component parts have been liberated, and thus rendered easily recognisable.

These facts are completely analogous to the observations which I have previously communicated when discussing Kyes' researches on snake venom. I even think it probable that the sugars may, in a similar manner, be chemically firmly connected with the cells, and in like way the complex carbohydrates, *e.g.*, glycogen, may well be present in the body in a hidden form. That is at least made probable by an observation of mine on finding glycocen in the polynuclear cells, viz., that the normal polynuclear leucocytes never show the characteristic reaction with iodine, whilst it is fre-

quently observed if some injury has befallen the body. Thus, as you are aware, in a number of intoxications the leucocytes give a positive glycogen reaction. Even the slightest stimuli may cause the leucocytes to split off glycogen in their protoplasm.

It will be necessary to assume that all these compounds are joined to certain protoplasmic groups, and that the decomposition of these compounds is the result of a fermentative action, resembling the decomposition of amygdalin, under the influence of emulsin, into glucose, hydrocyanic acid and benzaldehyde. Further, it is very interesting to note that one can often, by simple extracting substances like alcohol or ether, remove from the cells fat in the form of substances which still contain a protoplasmic component, although chemically they would appear at first sight to be pure lipoid substances. Such extracts have been prepared from red blood corpuscles by Landsteiner, Bang and Forssmann, who found that by injecting such extracts into animals one can obtain specific hæmolysins. This phenomenon, which they endeavoured to use as an argument against my side-chain theory, is explained in the *simplest* manner by Kyes' observations upon

the snake-venom lecithide. This lecithide would appear to be a pure fatty substance, being soluble in chloroform and even in toluol and alcohol, but as a matter of fact it contains, as I was before able to show you, a very slight quantity of the poison in chemical combination. And it is this portion which induces the biological reaction. The fact that in spite of this component being of a non-fatty nature, the compound apparently behaves like an ordinary fat, is due to the presence in it of a great number of fatty molecules which have given to the whole substance their specific characters.

I have frequently laid stress upon the fact that many nutrient substances are not present in the cells in a free state, and that, therefore, they are not at the disposal of any invader, but that a struggle is always necessary before they can be rendered accessible. In the case of snake venom I have shown that we are dealing with a simple difference between two chemical avidities. In the case of bacteria the affair may be rather more complicated, according to the occurrence of either of two possibilities, viz.: (1) A direct assimilation in consequence of a higher degree of affinity, or (2) an indirect action by injury to the cell. Supposing, for

example, that a microbe had penetrated into a cell, but had not the power of directly splitting off the fatty bodies; then these substances would not be immediately assimilable by it. Yet the microbe might obtain possession of them if it secreted a poison injurious to the cell, which would decompose the protoplasm. In such a case the fat would be liberated, and could be assimilated by the micro-organism, even though the immediate affinity between the two were very low. At any rate, these considerations may so far be of interest as they touch upon an issue of biology and pathology, which has, on the whole, been somewhat pushed into the background of late years, since one has accustomed oneself in every infective process to think in the first place of a specific bactericidal action through the direct toxic substances of the body, its haptines, etc. This has led to our neglecting somewhat the simple possibility that certain micro-organisms may only be able to grow if certain conditions are given for their development, and that they must perish if these conditions are withheld. To my mind this possibility is of importance in many respects, and is especially significant for *our views* on cancer immunity. At any rate,

we are dealing in these questions with a highly complex field of research, in which many possibilities are present, the most important being this difference of avidities.

We have previously seen that the trypanosomes may alter their avidity for arsenic, and thus acquire an apparent immunity. The contrary may occur in the case where the substance in question is not a poison, but a nutritive body. Here we should be perfectly justified in supposing the avidity of the receptor to have become increased. The same thing may take place with such micro-organisms which can physiologically diminish or increase their avidities. Thus in all these cases the struggle lies between the adaptability of the parasite and that of the host. The one whose adaptability is the highest will remain the victor.

Of course this struggle is to a great extent influenced by indirect actions, consisting in the secretion by each antagonist of dissolved substances hostile to the vitality and receptivity of the other organism. On the part of the bacteria, these substances are the toxins and the dissolved intracellular substances; on the part of the body, the anti-substances.

A further *rôle* is played by actions of a pro-

tective and defensive nature. Thus we have shown that bacteria congregate in those parts where they find the most favourable conditions of nutrition, whilst the organism, both by its phagocytes and by means of encystment, endeavours to render the pathogenic germs harmless and to eliminate them. You see, therefore, that this is a war waged in different fields, but in which to every action there corresponds a reaction. The war is waged in a three-fold manner—by variations of affinity, by variations in the poison, and by localisation.

LECTURE III.

Chemo-therapeutic Studies on Trypanosomes.

The studies on trypanosomes, which have of late been carried out with the greatest energy, especially in the Institut Pasteur in Paris, the School of Tropical Medicine in Liverpool, and the Speyerhaus in Frankfort-on-the-Main, are of the greatest theoretical and practical importance; for in this field of study the patient research work of Experimental Therapeutics has first come into full activity, and at the same time a basis has been gained on which we may hope successfully to undertake the practical attempt of suppressing sleeping-sickness. The exceedingly great difficulty of these studies is evidenced by the fact that hundreds and thousands of substances have to be examined by animal experiments, before a few producing a therapeutic effect can be found. I myself have in course of time examined more than 600 such substances. From their effect on animals, hints

may be obtained as to what to avoid, and in what direction therapeutics should advance. I will not enter here into the details of my work, but will only give you a general *résumé* of the chief results.

The substances which have hitherto proved efficient in combating trypanosome infections can be divided into three groups, viz. :—(1) The group of the basic triphenyl-methane dyes; (2) The group of the benzidine dyes; (3) The group of the arsenicals.

The salts of mercury may be used as adjuvants to each of the foregoing.

Among the triphenyl-methane stains, which were first employed by Wendelstadt, parafuchsin is by far the most satisfactory. It is possible, by prophylactic feeding with this drug, to render mice resistant to trypanosome infections for a long time, and I believe that this innocent substance might well be employed in the Tropics for the prophylaxis of human sleeping-sickness.

As regards the second type of substances, belonging to the benzidine group, trypan-red is a cotton dye which shows the remarkable property of staining mice an intense red, which *remains* fast for months. I have also prepared

a number of similar dyes belonging to the benzidine series, some of which possess an even more powerful action than trypan-red. In several cases a blue dye belonging to the same group, which was prepared by Nicolle and Mesnil, has proved still more efficient.

The value of arsenic acid which has for a long time been employed in the treatment of trypanosome diseases was first recognised by Laveran. Later on it was discovered, at the Liverpool School of Tropical Medicine, that atoxyl was a far more powerful substance. It is prepared by treating aniline with arsenic acid. The makers believed it to be an anilide of arsenic acid, *i.e.*, a compound in which the arsenic acid radical is supposed to be attached to the amido group in a similar manner as the acetic acid is present in acetanilide. Now, as you all know, the ammonia radical, which in aniline is very ready to react, has completely lost this property in acetanilide. This latter body is in fact almost an indifferent compound. Similarly, by the view that atoxyl was the anilide of arsenic acid, any further development of this compound was rendered impossible. I was, however, able to prove, together with Dr. Bertheim, that this compound is to be

regarded as something entirely different, namely, that the amido group is quite free and that the arsenic acid radical is attached to the benzol ring in the para position. Atoxyl must, therefore, be regarded as the sodium salt of paramido-phenyl-arsinic acid.

We have obtained a great number of substances derived from it, and have tested them therapeutically. Thus we found that, *e.g.*, by the introduction of the acetyl radical into the amido group the acetyl-amido-phenyl-arsinic acid is produced, which is far less toxic for mice than atoxyl. By means of this substance it is even possible to solve one of the most difficult therapeutic problems imaginable. For mice that have been infected with our most virulent strains, and which, without treatment, would die within three days, can in two-thirds of the cases be saved by this substance if it is given twelve to fourteen hours before death. Such curative results have never yet been published; for in previous communications there were mentioned almost exclusively experiments carried out at earlier periods of infection. In our cases acetyl-atoxyl cures mice in nearly 100 per cent. Unfortunately, acetyl-atoxyl is decomposed *in other* animals, especially in the horse,

into acetic acid and aniline, and it cannot, therefore, be nearly as effective as it is in the mouse. Nevertheless, the results obtained in the mouse are so excellent that they must encourage us to energetically pursue the path of these discoveries.

I mentioned in my last lecture that it is possible to obtain strains of trypanosomes which are resistant to these active substances. Studies, which I have carried out with Dr. Röhl and Dr. Browning, have shown this resistance to be of a very high degree. Thus, atoxyl-fast strains show no sign of being influenced by the highest doses of atoxyl which are applicable to mice. I gave you the explanation for this resistance, viz., that it is the result of a decrease in the avidity of the trypanosomes for these trypanocidal substances. Further, I pointed out that, apart from this resistance, the parasites' protoplasm, although not their nuclei, had become hyper-sensitive towards the arsenical preparation. Thus we meet with an important instance of association, in one and the same cell, of resistance and hyper-sensitivity. This association, which is of late years proving to be of increasing importance in the question of mammalian immunity, is therefore present

even in so simple an organism as this protozoon.

Although in the mouse atoxyl-resistance usually develops only after a fairly long period, I have occasionally seen it after a fortnight. This observation is very important, because it makes it possible that the negative results observed by Ayres Kopke, Broden, Rodhain, Todd and van Campenhout, in cases of sleeping-sickness which had received a long-continued treatment, may also be due to such a resistance.

In the same way as against atoxyl, trypanosomes can also be rendered resistant to parafuchsin, trypan-red and trypan-blue, and further, it was shown in my Institute by Franke in his work with apes, that it is even possible for trypanosomes to become resistant to those protective substances which are formed in the animal after its recovery from a trypanosome infection. A similar discovery has recently been made by Levaditi for the spirochætes of relapsing fever. This is therefore a general law, and it is especially important, because this change in the trypanosomes, having once become acquired, remains an hereditary property. Thus I have cultivated for 125 generations my atoxyl-*fast strain,* without finding any decrease in its

power of resistance. We must further note that every such resistance is specific. Thus the fuchsin-fast strains are resistant only to this substance, but not to atoxyl and trypan-red, and *vice versâ*. This resistance extends, however, to chemically different substances of the same group, so that we can in any individual case decide to which group a given new trypanocidal agent belongs. Thus, *e.g.*, the atoxyl-fast strain is resistant also to a number of related substances. Among hundreds of trypanocidal bodies, it is thus possible to single out those substances which contain arsenic.

It has further been shown that the resistance to trypan-red and trypan-blue is a mutual one, in spite of considerable chemical differences between these two substances, which in fact have nothing in common with one another except that they contain naphthalin rings, which are substituted in a certain position (3, 6) by sulphuric acid radicals.

Thus we possess in this specific resistance, so to speak, a therapeutic sieve, a *cribrum therapeuticum*, with which we can undertake the classification of any new chemo-therapeutic substance. If such a substance is found to have a destructive effect on our three different resistant strains,

it necessarily belongs to a fourth chemical group. One can, of course, materially simplify this examination by employing strains that have been rendered resistant to all the groups hitherto known. Thus I have, together with Dr. Browning, obtained a strain which is resistant to atoxyl, trypan-red and fuchsin. By using such a strain, a single animal experiment suffices for the purpose of establishing any new group of such chemo-therapeutic agents. For, if one injects the substance in question to such a trebly resistant mouse, the trypanosomes will either go on proliferating—then the substance must belong to one of the three known groups —or the trypanosomes are injured; in that case we have before us a new type. As you see, we are therefore enabled by means of these resistant strains to separate substances of different modes of action from one another, and thus to solve a problem which hitherto could not even be suggested. A way is thus shown us by which we may enter upon the consideration of the most intricate problems of pharmacodynamics and, if I may say so, obtain a fuller knowledge "*de sedibus et causis pharmacorum.*"

I also wish to lay especial stress upon my *view that the* drugs, also, are attracted by and

bound to the protoplasm molecule by certain atom groupings. I am inclined to look upon this as somewhat analogous to the binding of the toxins and of similar proteid bodies. Yet on the other hand, there are fundamental differences between the two. For, as I have always insisted, the mode of binding the toxins is peculiar in so far as it is the result of a certain kind of assimilation which obviously consists in processes of a more or less synthetic nature. These toxin-receptors which produce immunity are bodies of a more independent character, and appear to be especially destined for purposes of assimilation. This high degree of independence is evidenced by the fact that, in conformity with my side-chain theory, these receptors are very easily reproduced by the cell in excessive numbers, and after being separated from the cell, find their way into the blood.

I have now formed the opinion that in like manner a part of the chemically defined substances is attached to the cell by groups corresponding to these receptors; these atom groupings I will distinguish from the toxin-receptors by the name of "chemo-receptors." This view is more particularly supported by the fact before mentioned, that the atoxyl-fast

strain is also resistant to a number of substances related to atoxyl, but otherwise showing widely different chemical characteristics. Evidently, therefore, the arsenic acid radical here represents the point of attack which is common to this series of substances. It is the arsenical radical as such, which is bound by the chemo-receptors. These chemo-receptors, must, however, be regarded as of a simpler structure than the toxin-receptors. They do not show a similar degree of independence; they cannot therefore become increased in chronic intoxications, and since they are sessile, they cannot be thrown off into the blood. The number of such chemo-receptors for poisons which a trypanosome cell possesses, represents the number of points of attack. By means of the resistant strains we can count off one by one these groups that are open to attack.

The discovery of any trypanocidal substance will always be the result of chance and experiment. Every point of attack may, of course, be liable to be attacked by a host of different bodies, all of which have one specific group in common. It must be the goal of our experiments to search, by systematic chemical researches, for *the most* suitable substances, and thus to dis-

cover the "optimum centres." This problem is by no means an easy one, since according to my experience of many years, which agrees entirely with that of Mesnil, different animal species have different optima, and therefore the work must be specially done for each species of animals and each kind of trypanosomes. Thus, *e.g.*, in the mouse, *Trypanosoma gambiense* is far more easily attacked by arsenical preparations than the other strains of animal-pathogenic trypanosomes, whilst fuchsin and its relatives show an exactly opposite behaviour. Still, in spite of all these difficulties, the problem is not insoluble, since there are only a limited number of animal species and of trypanosomes; we may, therefore, confidently cherish the hope that the united forces of the observers who are at work on this subject in various laboratories will ultimately succeed in attaining the victory over sleeping-sickness, which is practically the most important.

Especially with a view to practical therapeutics the knowledge of the different points of attack is necessary, since it furnishes us with the possibility of fighting the disease by the combination of several chemo-therapeutic agents.

The principle of this combinatory method of therapeutics is, to attack the enemy simultaneously from two or three sides, and thus to produce a completely successful result by the combination of several different substances, each of which alone does not show a sufficient effect. Thus it has been found by Laveran and by Franke that certain infections which are influenced neither by trypan-red nor by atoxyl alone, can be cured if these two substances are used in conjunction. I, too, have in course of time repeatedly met with similar cases, and am convinced that substances which by themselves are not very efficient, may yet produce good results if they are used as adjuncts to those more powerful, though not completely curative, substances. Supposing, *e.g.*, a highly trypanocidal remedy to kill ninety-eight among one hundred parasites, its administration would be followed by an instantaneous improvement, but not a definite cure, since the two surviving trypanosomes must in course of time produce a relapse. But if one combined this powerful remedy with another which, even though much weaker, yet sufficed to kill just those two surviving but attenuated parasites, then such a combination *would serve* to bring about the otherwise un-

attainable cure. In this respect a publication from the Liverpool School of Tropical Medicine is most interesting, as it demonstrates the successful result of the employment of supplementary doses of perchloride of mercury in the atoxyl treatment of animals infected with sleeping-sickness.*

For man the combinatory treatment is of the greatest importance for a further reason. The complete sterilisation of the body, *i.e.*, the total destruction of every parasite, is often only to be accomplished by the administration of doses which come very close to the lethal dose. Such a procedure directly endangering life may be permissible in the animal experiment, but never in man. One is, however, justified in hoping that it might be possible to cure the disease without endangering life by the simultaneous administration of three, four or five substances, chosen in such a manner that their actions are concentrated on the parasites, whilst in the organism of the vertebrate host they are distributed over several different organs.

It is only natural that modern therapy which

* The perchloride in these cases has only the effect of an *adjuvant*, since it has been shown that its action on the trypanosomes, if administered alone, is quite inadequate.

is, as a rule, brought to bear upon man under circumstances making it very difficult to judge correctly of the processes occurring during treatment, has in the last decades worked chiefly with simple bodies. On the other hand, I would remind you that in the prescriptions of the physicians of the middle of last century, a predilection for long and complex prescriptions was evident; and although nowadays this predilection may appear to us obsolete, yet it must surely have sprung from a good power of observation, enhanced by rich personal experience. Although this former practice of therapeutic procedure may have overshot the mark, it would yet appear to contain a nucleus of truth, and it has certainly received the approval of science through these recent discoveries.

The importance of combined treatment is shown, lastly, in the phenomena of resistance already discussed. The fact that by frequently repeated administration of not completely sterilising doses, there is gradually acquired a resistance to the substance in question, makes it especially desirable that the first attack should be as complete as possible. This object, from what I have previously said, may probably best *be carried* out by a suitable combination of

substances. I would like here to call your attention to the fact that two decades ago I discovered a considerable efficacy of methylene blue against certain forms of malaria. This dye has not, however, obtained any extensive employment, since its effect is inferior to that of quinine. On the ground of our recent experience one would, I think, be perfectly justified in attempting to re-inforce the attack on the malaria parasites by employing combined doses of quinine and methylene blue.

Having now reached the end of my lecture, will you allow me once more to call your attention to the fact that the importance of these trypanosome studies is a twofold one; first, for theoretical reasons, because they afford us a deep insight into the finest mechanism of the action of the drugs; and second, for practical reasons, since by the methods of research employed and especially by the development of the combined treatment, the way has been prepared for a successful fight against trypanosome diseases. At the same time we are also justified in hoping that even beyond these narrow limits the studies may prove generally fruitful in the great campaign against infectious diseases.